Soil & Tillage Research 209 (2021) 104932

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Does crop rotation affect soil organic matter stratification in tillage systems?

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ARTICLE INFO

Keywords:
Crop diversity
Carbon chemistry
Conservation tillage
Perenniality
Triplett-Van Doren

ABSTRACT

Tillage practices are known to influence soil stratification, but crop rotation can strongly affect fluxes of energy and matter, and thus play a role in determining soil organic matter (SOM) fate. Using a high-resolution stratified soil sampling at 2.5 cm intervals from 0 to 30 cm depth, we evaluated the half century impacts of tillage and crop rotation on SOM stratification, and how specific soil organic functional groups drive SOM accrual in typical USA Midwest agroecosystems. Three levels of tillage intensity—no-tillage, chisel, and moldboard, and three crop rotations—continuous-corn, 2-year corn-soybean, and 3-year corn-forage-forage—were evaluated at two sites with contrasting soil characteristics (silt-loam versus clay-loam). Soil organic matter concentration was determined by loss on ignition and soil organic functional group abundances were estimated by diffuse reflectance infrared Fourier transform spectroscopy (mid-DRIFTS). No-tillage systems had a more stratified distribution of SOM than in more intensive tillage systems, with greater SOM concentrations in the uppermost layers of silt-loam (0−10 cm, 9.4–20% change to chisel/moldboard) and clay-loam soils (0−30 cm, 12–15% change). Under no-tillage, crop rotation with forage maintained or increased SOM accrual (0–17.5/20 cm, -0.9–22% change to other rotations), but including soybean in the rotation diminished SOM accrual when compared to continuous-corn (-8 to -12 % change) or corn-forage rotation (-11 to -18 % change). Abating tillage increased abundance of aliphatic and phenol in the uppermost soil layers, and these organic functional groups were primarily driving SOM accrual, while SOM was inversely related to more recalcitrant functional groups (aromatic, carbonyl, and carboxylate). Soil organic functional groups (65.4 % of R^2) and soil type, sampling depth, and management (34.6 % of R^2) accurately predicted SOM concentrations (R^2 = 93.4) underscoring their importance as a pathway to SOM accrual. Minimizing tillage intensity and rotating perennial forages in corn-based agricultural systems may lead to greater organic resource abundance that drive SOM accrual. This may present additional benefits to soil health, plant roots, and soil organisms in conservative agricultural systems.

1. Introduction

The non-uniform vertical distribution of properties in a soil profile (also known as stratification) results from natural processes of soil formation and anthropogenic factors that can change this pattern. Tendency towards soil stratification is maintained in agroecosystems when soils are not tilled. Tillage operations de-stratify the plowed layer by inverting, loosening, mixing, and/or breaking-down soil, and the magnitude of soil disturbance is determined by the tillage intensity. Though counterintuitive to many agriculturalists (Grove et al., 2007), soil stratification tends to favor plant roots (Qin et al., 2004; Micucci and Taboada, 2006; Schenk, 2008; Costa et al., 2010; Farmaha et al., 2012; Li et al., 2017) and soil organisms (Fierer et al., 2003; Eo and Nakamoto, 2008; Hsiao et al., 2018) by increasing resources availability closer to the soil surface; where roots and organisms are generally in greater abundance even in tilled soils. The degree of soil stratification will depend on management decisions and environmental factors; however, effects of crop diversity on soil nutrients and carbon (C) remain poorly understood (Isbell et al., 2017), notably in regards to the inclusion of perennials in the crop rotation. Introducing new species to a cropping system can change below- and above-ground biomass inputs and explore the soil matrix with distinct root architectures, thereby altering the

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https://doi.org/10.1016/j.still.2021.104932
Received 1 June 2020; Received in revised form 23 November 2020; Accepted 29 December 2020
Available online 8 February 2021

habitat for soil organisms and fostering new paths for soil organic matter (SOM) stabilization.

Understanding how agricultural management affects the stratification of SOM and its composition may allow substantiating agricultural practices to improve soil health and consequently agroecosystem functioning. Increasing organic matter in agricultural soils can enhance a myriad of soil biological processes (Angers and Caron, 1998; Watts et al., 2001; Schulz et al., 2013; Wagg et al., 2014; Lange et al., 2015; Barrios et al., 2018; Kravchenko et al., 2019) that ultimately lead to greater resilience against environmental shocks (Griffiths et al., 2000; Peterson et al., 2018). The magnitude and direction of processes driving SOM accumulation depends to some extent on the organic matter composition (Lutzow et al., 2006). Soil organic matter accrual is primarily determined by the amount and chemistry of organic matter inputs and soil biological activity, and environmental factors regulating interactions among those. Recent studies have shown that chemically complex, recalcitrant soil C functional groups (e.g., aromatics), do not necessarily dictate SOM stability or persistence (Lorenz et al., 2007; Schmidt et al., 2011; Lehmann and Kleber, 2015), but instead chemically labile C, such as aliphatic functional groups, contribute a greater extent to the formation and stabilization of SOM (Lehmann et al., 2007; Cotrufo et al., 2013; Hernandez-Soriano et al., 2018; West et al., 2020). What remains to be explored is how tillage and crop rotation affect the stratification of these organic functional groups and how they contribute to SOM accrual.

Soil organic matter vertical distribution and composition in the soil profile are known to be affected by tillage systems, but the effect of crop rotation is less understood. This is especially true in regard to high-resolution stratified soil sampling and long-term effects of tillage and crop rotations. Therefore, the objectives of this study were to i) evaluate how > 50 years of tillage and crop rotation affect SOM stratification and ii) evaluate how specific soil organic functional groups contribute to SOM accrual. Using a stratified soil sampling at 2.5 cm intervals to a depth of 30 cm, three levels of tillage intensity—no-tillage, chisel, and moldboard, and three crop rotations—continuous-corn, 2-year corn-soybean, and 3-year corn-forage-forage—were evaluated at two sites with contrasting soils (silt-loam versus clay-loam).

2. Materials and methods

2.1. Sites and soils

Sites were the Triplett-Van Doren Tillage and Crop Rotation Experiment started in 1962 near Wooster, Ohio, USA (40°45′ N, 81°54′ W) and in 1963 near Hoytville, Ohio, USA (40°45′ N, 81°54′ W) (Dick et al., 2013).

At Wooster, the dominant soil series is the Wooster silt loam (fine-loamy, mixed, active, mesic Oxyaquic Fragiudalfs). The parent material is low-lime glacial till, with a sporadic loess mantle of up to 51 cm thickness, and contains a fragipan at a depth ranging from 45 to 100 cm. This silt loam is well drained, having moderate permeability above the fragipan and moderate to slow permeability in the fragipan (USDA-SCS, 1984), with a slope ranging from 2 to 6 %, and a low to no shrink-swell potential (Soil Survey Staff, 2013a). Soil particle size (texture) distributions (0−30 cm) were between 21–25 % for sand, 60–61 % for silt, and 15–18 % for clay (Dick et al., 1986a). Soil pH (1:1 soil weigh to water volume ratio) ranged from 5.4–6.8 (0−30 cm). Native vegetation was hardwood forest (red, white and black oak) and relic forest remnants occur along creeks and in small woodlots (USDA-SCS, 1984).

At Hoytville, the dominant soil series is the Hoytville clay loam (fine, illitic, mesic Mollic Epiaqualfs) developed on glacial-lacustrine deposits (glacial till reworked by wave action on a nearly lake plain level) (USDA-SCS, 1973). This clay loam is a very deep, poorly drained soil, with a slope ranging from 0 to 1 %, and high shrink-swell potential (Soil Survey Staff, 2013b). Subsurface tile drainage was installed in the Hoytville site nine-years before the experiment started, with 10 cm inside-diameter tile drains placed at 17 m spacing and 1.2–1.4 m depth (Dick et al., 1986b). Soil particle size (texture) distributions (0−25 cm) were between 16–21 % for sand, 38–42 % for silt, and 37–46 % for clay (Dick et al., 1986b). Soil pH ranged from 4.3–7.5 (0−30 cm). The original vegetation was a deciduous swamp forest (USDA-SCS, 1973).

Both locations have a humid continental climate, with a mean annual air temperature of 10.7 and 9.9 °C and average annual precipitation of 874 mm and 1018 mm at the Hoytville and Wooster sites, respectively (Arguez et al., 2010a, 2010b). More details about agronomic practices are described below and more information is available in the Triplett-Van Doren Tillage and Crop Rotation website (https://kb.osu.edu/handle/1811/55716).

2.2. Experimental design and treatments

The experiments were a two-way factorial design with three levels of tillage intensity and three crop rotations in a randomized complete block design with three replications. Tillage systems were (1) no-tillage, where the residue from previous years' crops are left on the field and a single slot opening is used during planting; (2) chisel, a minimum tillage using a paraplow (1962–1983) and a chisel plow (1984-present) to loosen the soil while leaving > 30 % of the previous year's residue at the soil surface; and (3) moldboard, a plow tillage where a moldboard plow was used to invert the soil to a depth of about 20 cm, thus burying most of the residues. Historically, tillage has been conducted during spring in the Wooster silt loam and during fall in the Hoytville clay loam. Secondary tillage operations occur in moldboard and chisel systems. Crop rotations are (1) continuous-corn (*Zea mays* L.); (2) 2-year corn and soybean (*Glycine max* L.) rotation; and (3) 3-year corn and oat (*Avena sativa* L.) and/or alfalfa (*Medicago sativa)* or clover (*Trifolium repens* L.) rotation. At harvest, crop residue was left in the field for corn, soybeans, and oats, while alfalfa and clover were cut for hay typically 2–3 times a year. With minor modifications over the years, these treatments have been continuously maintained since their beginning in 1962 (Wooster) and 1963 (Hoytville) (Dick et al., 2013). The Wooster experimental unit or individual plot dimensions were 22.3 m by 4.3 m, while in Hoytville experimental units were 30.5 m by 6.4 m.

2.3. Soil sampling

Soils were sampled in late October 2014 after corn harvest at each site. At each experimental unit, ten soil cores from 0 to 30 cm depth were sampled, using a 2.5 cm diameter soil push probe. Each core was divided into 2.5 cm intervals with a clean knife and then the corresponding depth increments from each of the 10 cores were composited for a total of 12 depth increments per plot. Therefore, at both sites there were 9 treatments (3 tillage treatments × 3 crop rotations), and 12 depths, replicated 3 times each for a total of 324 soil samples (648 total samples across both sites).

2.4. Soil analyses

After sampling, soil samples were air-dried, crushed, and made to pass a 2.0 mm sieve. Soil organic matter was measured by loss of weight on ignition in a muffle furnace at 360 °C for two hours (Nelson and Sommers, 1996).

Soil organic functional groups were estimated using diffuse reflectance infrared Fourier transform spectroscopy in the mid-infrared region (mid-DRIFTS). Soil subsamples were finely ground (Deiss et al., 2020a) and spectra were obtained using an X,Y Autosampler (Pike Technologies Inc., Madison, WI) coupled with a Nicolet iS50 spectrometer equipped with a diffuse reflectance accessory (Thermo Fisher Scientific Inc., Waltham, MA). Potassium bromide (KBr) was used for background spectrum collected at the beginning of each plate reading (i.e., every 23 samples). All measurements were conducted from 4000 to 400 cm^{-1}, 4 cm^{-1} wavenumber resolution, and with 24 co-added scans in absorbance mode (Deiss et al., 2020b). Four spectral readings were done on each soil sample (24 co-added scans each) to generate the spectral replicates that were further averaged prior to peak area analysis and predictions. The spectral readings were randomly located within a 3 mm diameter circle in the central position of each well configured in Auto-Pro™ software (Pike Technologies Inc., Madison, WI).

Soil organic functional groups were assessed by integrating peak areas using the local baseline technique, as described by Demyan et al. (2012). The local baseline is a virtual straight line added to the base of peak connecting the peak left and right limits. Local peak areas were determined in the absorbance spectra using the triangle method (R package 'geometry', Sterratt, 2019). Nine organic functional groups were systematically selected to cover different functionalities of SOM (Supplementary Fig. 1) and peak assignments as well as potentially overlapping inorganic functional groups are described in Supplementary Table 1.

2.5. Statistical analyses

Data was analyzed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). To test our hypothesis that soil management would affect SOM stratification, a three-way mixed-effects ANOVA ('lme4' R package, Bates et al., 2015) was used separately for each site, including SOM concentrations or specific organic functional group abundance (peak areas) as continuous dependent variables, tillage intensity, crop rotation, and soil depth as categorical fixed effects, and block as a categorical random effect. Significant interactions of tillage x soil depth or tillage x crop rotation x soil depth were evaluated ('stats' R package, R Core Team, 2016) and mean comparisons were based on least-square estimates using Tukey method with an alpha = 0.05 ('emmeans' R package) (Lenth, 2019). Soil organic matter was evaluated in two ways, i) the SOM concentration measured at each respective depth interval (e.g., 7.5 cm was the SOM concentration at 5.0–7.5 cm) and ii) the cumulative average SOM concentration, calculated as the average SOM from 0 to that respective depth. In other words, the cumulative average SOM at 7.5 cm depth was calculated by taking the average SOM concentration between the 0–2.5 cm, 2.5–5.0 cm and 5.0–7.5 cm intervals.

To test the hypothesis that soil organic functional groups contribute to modulate SOM accrual, the relationship between SOM concentration and organic functional groups abundance was evaluated using mixed-effect regression models ('lme4' R package, Bates et al., 2015). First, individual (bivariate) relationships between specific functional groups (peak areas) and SOM were tested separately for each site, including crop rotation nested within tillage as random factors. Secondly, all organic functional groups were combined to predict SOM concentration using multiple linear mixed-models regression. Organic functional groups abundances (peak areas) were treated as continuous fixed effects,

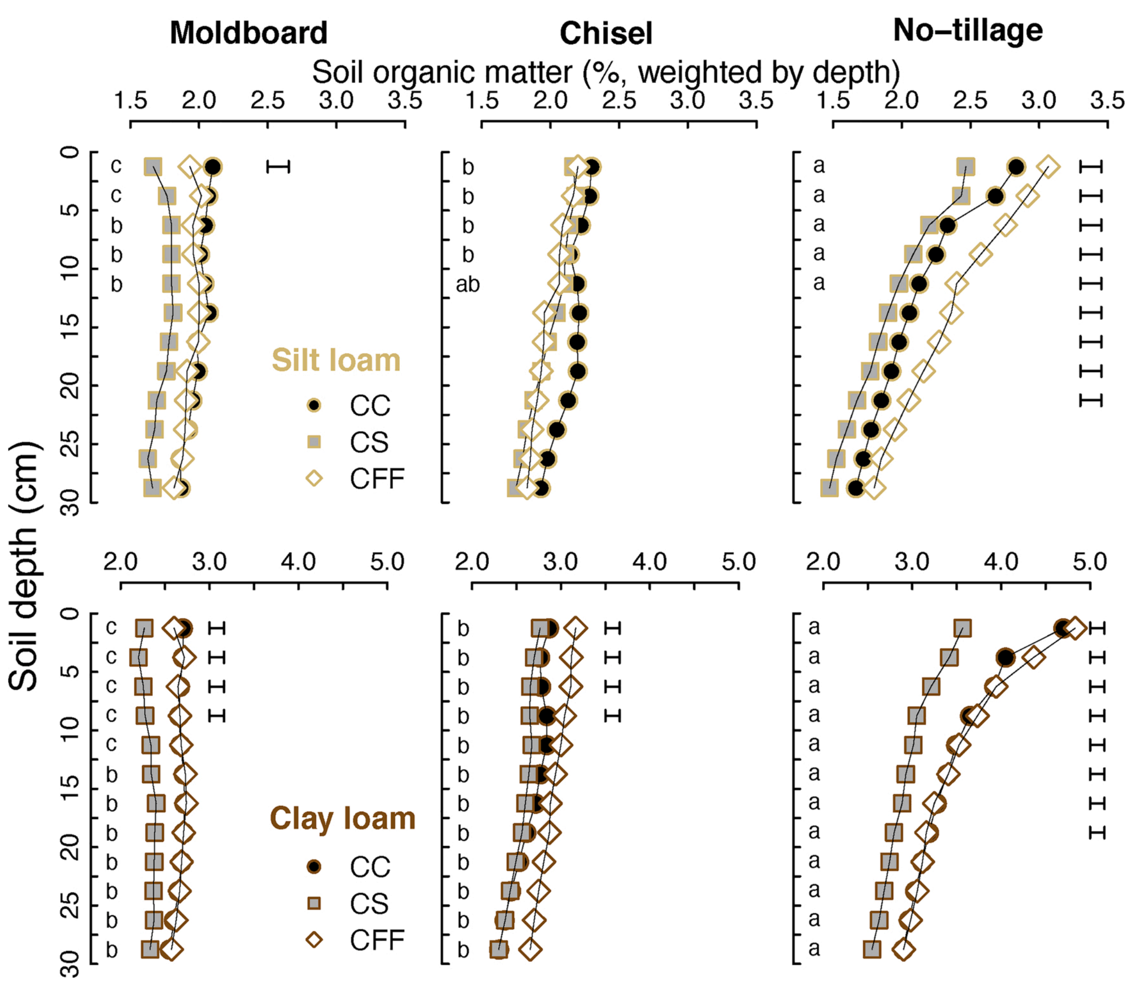

Fig. 1. Soil organic matter stratification (cumulative average concentration from zero to a given depth) under a gradient of tillage intensity (Moldboard, Chisel, and No-tillage) and crop rotation (CC: continuous-corn, CS: corn-soybean rotation, and CFF: corn-forage-forage rotation) at Wooster (silt loam) and Hoytville (clay loam). Letters indicate differences among tillage treatments across crop rotations at specific soil depths. Least significant difference bars separate crop rotations within a tillage treatment at specific soil depths (points separated at greater distances than the bar's size are different). Both comparisons were determined by the Tukey test (p <0.05).

and a four-way interaction among site, depth, tillage, and crop rotation was included as random effects. Model significance was determined using p-values from the F-test and variation partitioning was determined based on sum of squares ('stats' R package, R Core Team, 2016).

3. Results

3.1. Stratification of organic matter in the soil profile

Soil organic matter distribution in the soil profile was strongly affected by tillage, with decreasing stratification from no-tillage to chisel to moldboard systems (Fig. 1). Crop rotation affected SOM stratification (when calculated as the cumulative SOM concentration from 0 to each

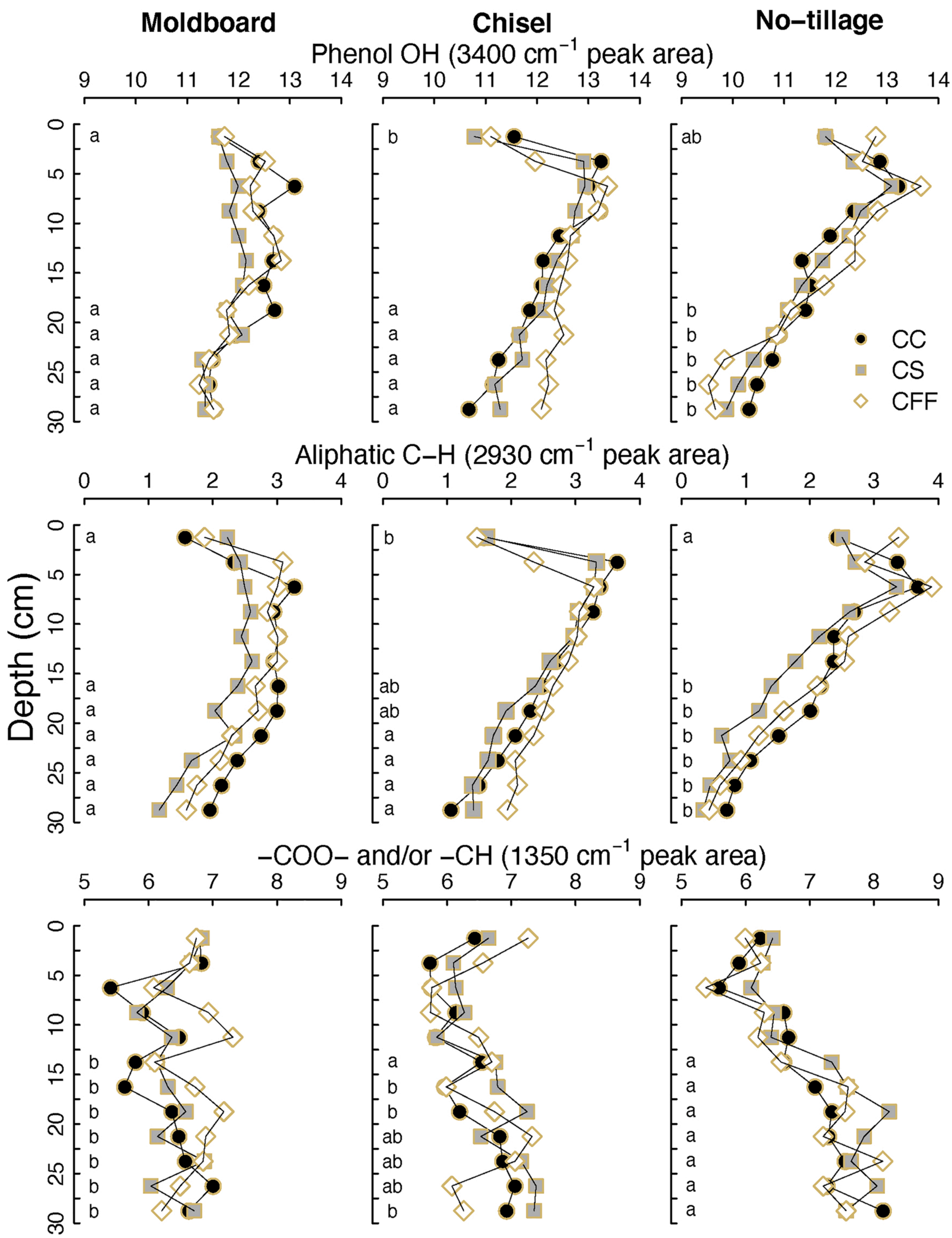

Fig. 2. Silt loam (Wooster) soil organic functional groups (mid-DRIFTS) stratification under a gradient of tillage intensity (Moldboard, Chisel, and No-tillage) and crop rotation (CC: continuous-corn, CS: corn-soybean rotation, and CFF: corn-forage-forage rotation). Letters indicate differences among tillage treatments across crop rotations at specific soil depths. Least significant difference bars separate crop rotations within a tillage treatment at specific soil depths (points separated at greater distances than the bars size are different). Both comparisons were determined by the Tukey test ($p < 0.05$).

respective depth) within tillage systems in both silt loam (Wooster) and clay loam (Hoytville) soils (Fig. 1). Most differences were found under no-tillage as crop rotation effects were strongly reduced in more intensive tillage systems (moldboard and chisel). In silt loam soils (Wooster) under no-tillage, corn-forage rotation increased SOM concentration down to 20 cm in relation to continuous-corn (12 % change) or corn-soybean rotation (22 % change). Corn-soybean rotation reduced SOM concentrations (0−20 cm) compared to continuous-corn (-8% change) or corn-forage rotation (-18 % change). In clay loam soils (Hoytville) under no-tillage, corn-forage had similar SOM concentration (0–17.5 cm) to continuous-corn (-0.9 % change), but corn-soybean rotation also reduced SOM concentration (0–17.5 cm) compared to continuous-corn (-12 % change) or corn-forage rotation (-11 % change).

Evaluating SOM stratification specifically at each depth (non-cumulative) (Supplementary Fig. 2), crop rotation changed SOM concentrations among tillage systems in silt loam soils (Wooster), but not in

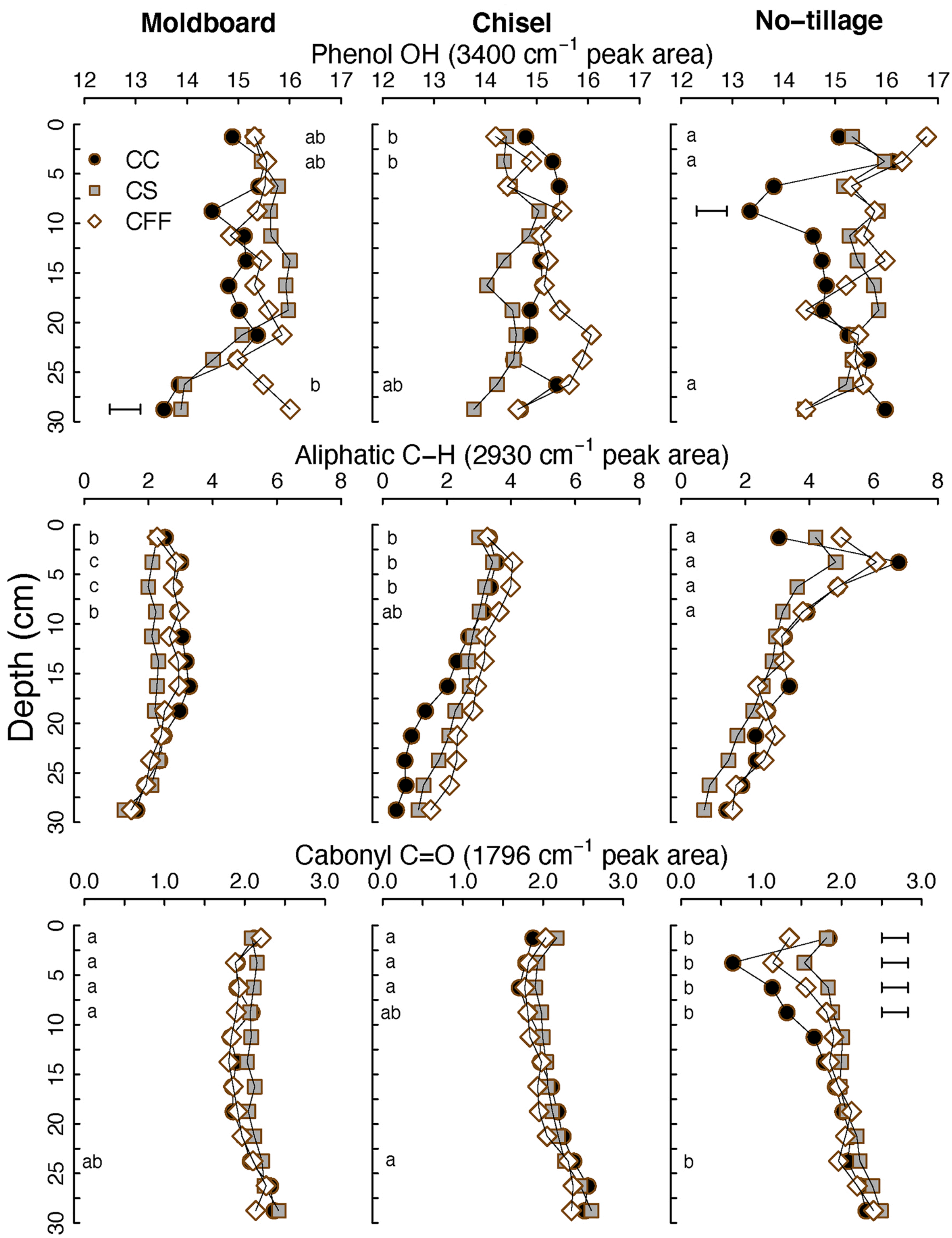

Fig. 3. Clay loam (Hoytville) soil organic functional groups (mid-DRIFTS) stratification under a gradient of tillage intensity (Moldboard, Chisel, and No-tillage) and crop rotation (CC: continuous-corn, CS: corn-soybean rotation, and CFF: corn-forage-forage rotation). Letters indicate differences among tillage treatments across crop rotations at specific soil depths. Least significant difference bars (points separated at greater distances than the bars size are different) separate crop rotations within a tillage treatment at specific soil depths. Both comparisons were determined by the Tukey test ($p < 0.05$).

clay loam soils (Hoytville). In silt loam soils, the corn-forage rotation had greater SOM than both continuous-corn and corn-soybean under no-tillage at soil depths 7.5 cm (49 % and 40 % change, respectively for rotations) and 15 cm (27 % and 44 % change, respectively for rotations), while the less diverse rotations (i.e, continuous corn or corn-soybean) had greater SOM at deeper soil layers under moldboard and chisel (top panels in Supplementary Fig. 2). Regardless of crop rotation, no-tillage increased SOM (non-cumulative in depth) in the uppermost layers of silt loam soils (Wooster) (0−5 cm) compared to chisel (21 % change) and moldboard tillage systems (37 % change), and clay loam soils (Hoytville) (0–7.5 cm) compared to chisel (25 % change) and moldboard systems (46 % change) (Supplementary Fig. 2). In contrast, more intensive tillage systems had greater or similar SOM concentrations in deeper soil layers than in less intensive tillage systems.

Stratification of soil organic functional groups (non-cumulative in depth) (Figs. 2 and 3) was only affected by crop rotation in no-tillage systems of clay loam soils (Hoytville) (Fig. 3). Under these conditions, corn-forage and corn-soybean rotations increased phenol (10 cm, 18 % and 19 % change, respectively) and carbonyl abundances (2.5−10 cm, 46 % and 70 % change, respectively) when compared to continuous-corn. Moreover, regardless of crop rotation, tillage intensity affected stratification of soil organic functional groups in both silt loam and clay loam soils (Figs. 2 and 3). There was a stratified distribution of soil organic functional groups in all tillage treatments, but to a greater extent in less intense tillage systems. The pattern of soil stratification was specific to each soil functional group (Figs. 2 and 3). For example, abundances of aliphatics (2930 cm^{-1}) decreased with depth (Figs. 2 and 3), while other functional groups such as carbonyl (1796 cm^{-1}) or carboxylate and/or aliphatics (1350 cm^{-1}) increased with depth. Interestingly, some of these functional groups had a V-shaped pattern of stratification closer to the soil surface (0 to ~5/10 cm, Figs. 2 and 3), which we attribute to greater heterogeneity of SOM composition in the uppermost soil layers (see below).

Irrespective of crop rotation in silt loam soils (Wooster), no-tillage had lower aliphatic abundance in deeper soil layers (non-cumulative in depth) when compared to moldboard (between 17.5 cm and 30 cm, -49 % change) and chisel (between 22.5 cm and 30 cm, -55 % change) (Fig. 2 and Supplementary Fig. 3). No-tillage systems had less phenol abundance (between 20 and 30 cm) than chisel (-11 % change) and moldboard (-10 % change), and greater abundance of carboxylate and aliphatics (between 15 and 30 cm) than chisel (11 % change) and moldboard (16 % change).

Clay loam soils (Hoytville) had a slightly different stratification of SOM functional groups when compared to silt loam soils (Wooster) regardless of crop rotation (Fig. 3 and Supplementary Fig. 4). No-tillage systems had greater aliphatic abundance (non-cumulative in depth) than moldboard (0−10 cm, 67 % change) and chisel (0–7.5 cm, 39 % change) (Fig. 3) under clay loam soils. No-tillage systems also increased abundance of phenol (0−5 cm) compared to chisel (8.6 % change) and moldboard systems (3.8 % change) and decreased abundance of carbonyl (0−10 cm) compared to chisel (21 % change) and moldboard systems (27 % change) (Fig. 3). Reduction in aromatic and/or carboxylate abundance was observed at 7.5 cm under no-tillage systems when compared to chisel (-15 % change) and moldboard systems (-20 % change) (Supplementary Fig. 4).

Heterogeneity of a selected soil organic functional group (aliphatics) was more pronounced in the near surface soil layers, especially under no-tillage systems (Supplementary Fig. 5 and 6). This impacted the characteristics of functional groups distribution among depths. No-tillage systems had the greatest heterogeneity of SOM composition closer to the soil surface (0–2.5 cm) in clay loam soils (Hoytville), where standard deviations among replicates increased by 77 % and 146 % compared to chisel and moldboard, respectively, and down to 5 cm in silt loam soils (Wooster), where standard deviations increased by 40 % and 116 % compared to chisel and moldboard, respectively.

3.2. Soil organic matter accrual

Soil organic matter functional groups composition modulated SOM accumulation (Fig. 4 and Table 1). Each soil type (silt loam or clay loam) had a specific SOM composition that drove SOM accumulation (Fig. 4), and a specific influence of tillage and crop rotation effects (Table 1). Soil organic matter concentration was positively related to aliphatic and to lesser extent phenol. Conversely, SOM concentration was inversely related or non-related (non-significant slope) to more recalcitrant carbonyl and aromatic and/or carboxylate.

The combination of all organic functional groups (Table 2 and Supplementary Fig. 7) accurately predicted SOM concentrations across both soil types (clay loam and silt loam), depths (0−30 cm, cumulative at 2.5 cm from zero to a given depth) and experimental factors (coefficient of determination [R^2] = 93.4, residual prediction deviation 3.69, and root mean squared error 0.16 %). These predictions were developed for the cumulative SOM concentrations (weighted by depth) (as presented in Fig. 1) and cumulative organic functional groups abundances (data not-shown). The organic functional groups had the greatest weight on the model (65.4 % of R^2) while the experimental sites, depths, and treatments contributed to a lesser extent (34.6 % of R^2). The only non-significant organic functional group was the Carbohydrate —COH and excluding it from the model slightly improved the AIC from 274 to 267, but it did not change the prediction accuracy. Only the reduced model is presented in Table 2.

4. Discussion

4.1. Stratification of organic matter in the soil profile

Soil management practices may be designed to take advantage of the ecological relations between soil, plants, and soil organisms, and set the path to increase agroecosystem productivity, resource use-efficiency, and resilience to environmental and anthropogenic shocks. In our study, abating tillage was associated with increasing both SOM concentrations (Fig. 1) and labile organic functional groups abundances (i.e., aliphatic) (Fig. 3) primarily in the uppermost layers of the soil profile. Such a top-down enhancement of SOM may increase use and cycling of soil resources following greater abundance and biological activity of plants and organisms near the soil surface (e.g., Fierer et al., 2003; Qin et al., 2004; Micucci and Taboada, 2006; Eo and Nakamoto, 2008; Schenk, 2008; Costa et al., 2010; Farmaha et al., 2012; Li et al., 2017; Hsiao et al., 2018). Increased SOM accrual is also related to improved soil health, reduced erosion risk, and enhanced soil C storage (Paustian et al., 2016; Lorenz et al., 2019). These improvements in the soil environment help building resiliency in agroecosystems facing adverse environmental conditions (Peterson et al., 2018).

Crop rotation played a major role in determining SOM accrual and stratification; however, benefits of increasing diversity were conditional to the crops included in the rotation and rotational effects were drastically reduced in more intensive tillage systems (Fig. 1). Under no-tillage, crop rotation with perennial forages maintained or increased (-0.9–22% change) SOM accrual (cumulative in depth) in both silt loam (0−20 cm) and clay loam soils (0–17.5 cm). Perennials that are part of these more complex rotations are known to produce greater root biomass than most grain crops (Syswerda et al., 2011; Sprunger and Robertson, 2018) and that can lead to increased SOM accumulation. Conversely, including soybean in the rotation decreased SOM accrual when compared to continuous-corn (-8 to -12 % change) and corn-forage rotation (-11 to -18 % change). These results confirm previous findings of reduced SOM (0−45 cm depth) in a corn-soybean rotation compared to continuous-corn (Huggins et al., 2007). This reduction is likely caused by lower biomass inputs and accelerated rates of SOM decomposition in soybean- versus corn-based systems (Huggins et al., 2007; Hall et al., 2019).

Not only greater stratification of soil organic functional groups

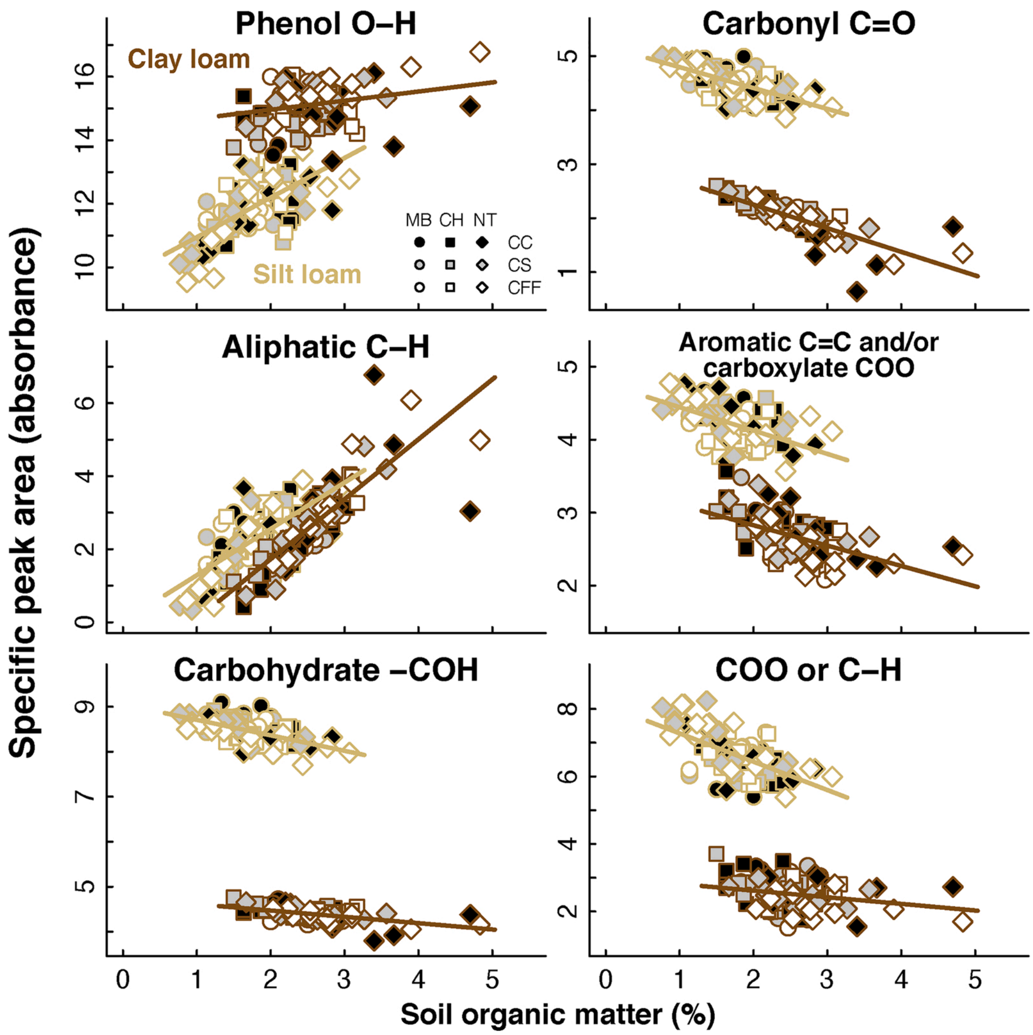

Fig. 4. Relationship between soil organic matter and organic functional groups (mid-DRIFTS) under a gradient of tillage intensity (MB: moldboard, CH: chisel, and NT: No-tillage) and crop rotation (CC: continuous-corn, CS: corn-soybean rotation, and CFF: corn-forage-forage rotation) at two sites with contrasting soil characteristics. Soil was sampled at 2.5 cm intervals to a depth of 30 cm. Regression predictions are indicated with solid lines and models coefficients are presented in Table 1.

occurred in no-tillage systems (Figs. 2 and 3), but also greater heterogeneity of these groups (standard deviation among replicates) in the uppermost soil layers (Supplementary Figs. 5 and 6). This heterogeneity created a V-shaped pattern of stratification for important soil organic functional groups (Figs. 2 and 3). In no-tillage systems, heterogeneity of aliphatics increased by 116–146 % compared to moldboard and 40–77 % to chisel across crop rotations in the top layers of silt loam (0−5 cm) and clay loam soils (0–2.5 cm) (Supplementary Figs. 5 and 6). Reduced soil disturbance and greater residue maintenance on/near the soil surface under various decomposition levels are main drivers of this heterogeneity. A heterogeneous SOM composition may benefit the coexistence of soil organisms with differing degrees of adaptation to accessing organic functional groups with distinct degrees of biological availability. Greater diversity of soil organisms is often found in soils under less intense tillage (Quadros et al., 2012; Smith et al., 2016; Wang et al., 2016) where diversity enables improved soil functioning through a variety of biological processes (Brussaard et al., 1997).

4.2. Soil organic matter accrual

After organic matter is added to the soil in various forms and at different depths as governed by tillage and crop rotation practices, SOM stabilization can occur through a number of processes. These include organic matter intrinsic chemical composition, specific interactions with mineral surfaces and metal ions (organo-mineral associations), and spatial inaccessibility to microbial decomposition as a result of physical occlusion within soil microaggregates or entrapment in small soil pores (Kiem and Kögel-Knabner, 2002; Six et al., 2002; Lutzow et al., 2006; Carrington et al., 2012; Barré et al., 2014; Kravchenko et al., 2019). In our results, organic matter chemical composition was indeed an important pathway to SOM accrual (Table 2 and Supplementary Fig. 7); however, even though chemical recalcitrance has been suggested as a mechanism of SOM stabilization, hypothesized C lability was the main driver in forming and stabilizing SOM (Table 1 and Fig. 4). Soil organic matter stabilization may be driven by the greater availability and/or affinity of these more labile functional groups, such as aliphatics, to form strong organo-mineral associations with reactive soil surfaces or to be physically protected inside pores or aggregates.

With lower SOM concentration (Supplementary Fig. 2) and lower abundance of labile functional groups (Fig. 2) in deeper layers of non-tilled soils, and therefore lower organic loading of mineral surfaces, there is a greater potential for developing new organo-mineral associations. The decline in the proportion of mineral surfaces complexed with organic compounds is often found in deeper soil horizons, except some podzolized soils (van Hees et al., 2000). We verified that moldboard and in a lower degree chisel increased SOM concentration in deeper soil

Table 1
Bivariate regression mixed-models statistical coefficients in the relationship between soil matter concentration and specific organic functional groups. Data is shown in Fig. 4.

Soil type (Location)	Peak	Soil organic functional group	Intercept	Slope	Total organic matter Relative proportion of R^2 (%)	Tillage intensity and crop rotation	Coefficient of determination (R^2)
Silt loam (Wooster)	3400	Phenol O—H	9.70	1.25	92.1	7.9	46.9
	2930	Aliphatic C—H	0.02	1.27	100.0	0.0	48.6
	1995	Carbohydrate —COH	9.05	−0.34	63.8	36.2	54.9
	1796	Carbonyl C═O	5.18	−0.39	90.3	9.7	50.9
	1620	Aromatic C═C or carboxylate COO	4.77	−0.32	76.3	23.7	35.2
	1530	Aromatic C═C	1.37	−0.08	35.3	64.7	19.9
	1350	C—H or COO	8.12	−0.84	82.6	17.4	45.3
	1159	C—O	0.91	0.01	27.0	73.0	16.4
	1022	C—O	7.04	0.69	100.0	0.0	18.3
Clay loam (Hoytville)	3400	Phenol O—H	14.39	0.29	25.1	74.9	28.6
	2930	Aliphatic C—H	−1.55	1.64	100.0	0.0	68.8
	1995	Carbohydrate —COH	4.75	−0.14	72.7	27.3	40.8
	1796	Carbonyl C═O	3.12	−0.44	100.0	0.0	61.7
	1620	Aromatic C═C or carboxylate COO	3.39	−0.28	68.8	31.2	44.2
	1530	Aromatic C═C	0.54	−0.03	100.0	0.0	1.9
	1350	C—H or COO	3.01	−0.20	41.4	58.6	17.3
	1159	C—O	0.85	−0.01	100.0	0.0	1.7
	1022	C—O	1.63	0.32	23.1	76.9	19.7

Table 2
Mixed-models multiple linear regression to predict total soil organic matter concentration (%, weighed by depth) and partitioning of variability in the relationships between soil organic functional groups and experimental factors.

Soil organic functional groups (t-values and signif. codes) [a]	Site, Depth, Tillage, and Crop rotation (variance ± std. error)	Fixed (peaks) (relative proportion of total variance %)	Random (experimental factors)	Coefficient of determination (R^2) Total variance explained (%)	RPD [b]	RMSE [c]	AIC [d]
PA_{3400} (11.6)*** PA_{2930} (6.8)*** PA_{1796} (-3.8)*** PA_{1620} (10.4)*** PA_{1530} (-5.7)*** PA_{1375} (-2.8)** PA_{1159} (12.8)*** PA_{1022} (-4.8)***	Interaction (0.11 ± 0.33) Residual (0.03 ± 0.19)	65.4	34.6	93.4	3.69	0.16	267.2

[a] p-value signif. codes: '***' 0.001 and '**' 0.01. PA: peak area and corresponding mid-infrared frequency (cm^{-1}).
[b] RPD: residual prediction deviation.
[c] RMSE: root mean squared error.
[d] AIC: Akaike information criterion.

layers (Supplementary Fig. 2) at expense of specific organic functional groups mechanically mobilized from the upper layers (Fig. 2). Intermediate products of decomposition such as aliphatics can escape further breakdown by forming organo-mineral associations with unloaded mineral surfaces, or by entrapment in small soil pores or micro-aggregates where they become inaccessible to microorganisms and their exoenzymes (Kiem and Kögel-Knabner, 2002; Six et al., 2002; Kravchenko et al., 2019). When soils are not tilled, however, increasing SOM accumulation in deeper soil layers may rely on enhancing below-ground biomass inputs from plants and soil organisms. Including perennial species in the rotation prime top-down SOM accrual (Fig. 1 and Supplementary Fig. 2); and optimizing perennials management with moderate events of grazing or cutting (Ferraro and Oesterheld, 2002; Pugliese et al., 2019) along with other agronomic practices such as nutrient management can enhance root growth. Ultimately, root tissue decomposition will contribute to biopore formation and organic matter addition as rhizo-deposits and necromass.

The temporal and spatial interactions between roots of diverse species may increase the proportion of the soil matrix covered with an interconnected network of pores of various sizes, creating an ideal micro-environment where microbes can process and transport organic compounds that can later be adsorbed onto soil mineral surfaces or entrapped in small soil pores and microaggregates (Kravchenko et al., 2019). Evaluating the same experiment as the present study, Burgos Hernández et al. (2019) found that non-tilled silt loam soils had a greater proportion of smaller pores < 30 μm at both 0–10 and 10–20 cm depths; pores which are associated with increased SOM persistence due to longer periods of anoxia that limits decomposition of more bioavailable organic compounds (Keiluweit et al., 2017). Moreover, unlike monocultures, diverse plant communities favor the development of 30–150 μm pores, and greater abundance of these pores translates into a greater spatial footprint that allows microorganisms to process and transport organic compounds to specific microsites where they can be stabilized (Kravchenko et al., 2019).

5. Conclusion

Crop rotation increases SOM accrual in corn-based agricultural systems when perennial forages take part in the rotation and soils are not tilled. These agroecosystems create a top-down pathway for SOM accumulation, and increase abundance of organic functional groups that drive SOM accrual. Crop rotation benefits are drastically reduced as tillage intensity increases and soybean is included in the rotation. Abating tillage and enhancing perenniality promote soil health in conservative agricultural systems.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

We recognize the pioneers Dr. Glover B. Triplett and Dr. David M. Van Doren who designed and established the experiment and Dr. Warren A. Dick who maintained it throughout the years. We thank Bethany Herman, Mason Gingery, Madison Campbell, and Noely Gonzalez-Maldonado for their support in the laboratory. We acknowledge Spectrum Analytic (Washington Courthouse, OH) who measured total organic matter on the soil samples. This research was supported by the Foundation for Food and Agricultural Research and the School of Environment and Natural Resources at Ohio State University.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.still.2021.104932.

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